SELECTIVE BLOCKADE OF MEMBRANE ATTACK COMPLEX FORMATION DURING SIMULATED EXTRACORPOREAL CIRCULATION INHIBITS PLATELET BUT NOT LEUKOCYTE ACTIVATION

Christine S. Rinder, MD[a,b]
Henry M. Rinder, MD[a]
Michael J. Smith, PhD[d]
Jayne B. Tracey, BS[a]
Jane Fitch, MD[b]
Lan Li, PhD[c]
Scott A. Rollins, PhD[c]
Brian R. Smith, MD[a]

Objective: **Complement activation is induced by cardiopulmonary bypass, and previous work found that late complement components (C5a, C5b-9) contribute to neutrophil and platelet activation during bypass. In the present study, we blocked C5b-9 formation during extracorporeal recirculation of whole blood to assess whether the membrane attack complex was responsible for both platelet and leukocyte activation.** ***Methods:*** **In a simulated extracorporeal model that activates complement (C3a and sC5b-9), platelets (CD62P expression, leukocyte-platelet conjugate formation), and leukocytes (increased CD11b expression and neutrophil elastase), we examined an anti-human C8 monoclonal antibody that inhibits C5b-9 generation for its effects on cellular activation.** ***Results:*** **Anti-C8 significantly inhibited sC5b-9 formation but did not block C3a generation. Anti-C8 also significantly inhibited the increase in platelet CD62P and monocyte-platelet conjugate formation seen with control circulation. Moreover, compared with control circulation, in which the number of circulating platelets fell by 45%, addition of anti-C8 completely preserved platelet counts. In contrast to blockade of *both* C5a and sC5b-9 during simulated extracorporeal circulation, neutrophil activation was not inhibited by anti-C8. However, circulating neutrophil and monocyte counts were preserved by addition of anti-C8 to the extracorporeal circuit.** ***Conclusions:*** **The membrane attack complex, C5b-9, is the major complement determinant of platelet activation during extracorporeal circulation, whereas C5b-9 blockade has little effect on neutrophil activation. These data also suggest a role for platelet activation or C5b-9 (or both) in the loss of monocytes and neutrophils to the extracorporeal circuit. (J Thorac Cardiovasc Surg 1999;118:460-6)**

The systemic inflammatory response to cardiopulmonary bypass (CPB) results in humoral and cellular changes contributing to clinical complications. Complement activation is an important contributor to the inflammatory response to CPB,[1,2] but the pathways linking specific complement components to secondary mediators are incompletely defined. Complement activation generates the proinflammatory and procoagulant moieties C3a and C5a and the membrane attack complex (MAC), C5b-9, whereas other neutral or anti-inflammatory components (eg, the opsonin C3b) are essential for normal immune function.[3]

In vitro studies have demonstrated a number of complement-mediated proinflammatory and procoagulant effects relevant to CPB. Generation of the MAC induces platelet α-granule release,[4] platelet prothrombinase activity,[5] and platelet expression of CD62P,[6] which mediates the binding of activated platelets to leukocytes.[7] We have demonstrated an increase in platelet CD62P during clinical CPB,[8] as well as a parallel increase in circulating monocyte-platelet conjugates.[9,10]

From the Departments of Laboratory Medicine[a] and Anesthesiology,[b] Yale University School of Medicine, New Haven, Conn; Alexion Pharmaceuticals,[c] New Haven, Conn; and Quinnipiac College,[d] Hamden, Conn.

Supported by National Institutes of Health grants HL47193 (B. R. Smith) and HL02668 (H. M. Rinder) and by Alexion Pharmaceuticals. C. S. Rinder is a recipient of an American Heart Association Clinician-Scientist Award.

Received for publication Jan 6, 1999; revisions requested March 4, 1999; revisions received April 21, 1999; accepted for publication May 14, 1999.

Address for reprints: Christine S. Rinder, MD, Department of Anesthesiology, Tompkins 3, Yale University School of Medicine, 333 Cedar St, PO Box 8051, New Haven, CT 06510-8051 (E-mail christine.rinder@yale.edu).

0022-5223/99 $8.00 + 0 **12/1/100084**

C5a formation up-regulates neutrophil (PMN) CD11b/CD18 through mobilization of intracellular granules,[11] a marker of leukocyte activation that we have demonstrated to occur on both PMNs and monocytes during clinical CPB.[10] Both C5a and C5b-9 also promote endothelial cell CD62P expression in vitro.[12]

In vitro recirculation of whole blood in an extracorporeal bypass circuit, for example, simulated extracorporeal circulation (SECC), has been used extensively to simulate platelet,[13] leukocyte,[14] and complement activation[15] on CPB. It serves as a preclinical model for examining the response of human blood to interventions targeting inflammatory or coagulopathic complications of CPB. Novel interventions blocking complement activation at different stages now allow us to selectively examine the pathophysiology of cell-mediated, complement-dependent responses to extracorporeal circulation. We[15] have previously demonstrated that simultaneous blockade of both C5a and the MAC prevented both platelet and PMN activation during SECC. In the current study, we used a blocking monoclonal antibody (Mab) directed against the complement component C8 to examine the specific role of the MAC on platelet and PMN activation during SECC.

Methods

Materials. A murine Mab directed against the human complement component C8 (clone 133.3) developed by Dr P. Sims was supplied by Alexion Pharmaceuticals (New Haven, Conn). Control Mab directed against mouse C5 (clone BB5.1) was the gift of Dr Brigitta Stockinger. All Mabs for fluorescence labeling were used as purified whole immunoglobulin G. Anti-CD41a recognizes platelet glycoprotein IIb/IIIa; this Mab and anti-CD62P were purchased from Pharmingen (San Diego, Calif). For leukocyte labeling, anti-CD45 (Pharmingen) and anti-CD11b (ExAlpha, Boston, Mass) were used.

In vitro studies[16] had demonstrated that the anti-C8 Mab at 30 μg/mL completely inhibited C5b-9 generation in human serum (measured both by a functional hemolytic assay and by enzyme-linked immunosorbent assay) without significant blockade of either C3a or C5a generation. Preliminary dosing studies indicated that anti-C8 at 40 μg/mL in whole blood was the lowest dose giving maximal C5b-9 blockade during SECC, and thus 40 μg/mL was used in the present study. In vitro whole blood experiments demonstrated that this dose of anti-C8 Mab had no direct effect on either platelet or leukocyte activation. The anti-C8 Mab did not inhibit platelet CD62P expression to a 1 μmol/L dose of epinephrine followed by a 5 μmol/L dose of adenosine diphosphate, nor the increase in CD11b on monocytes and PMNs in response to a 1 μmol/L dose of *N*-formyl-methionyl-leucyl-phenylalanine.

Extracorporeal circuit preparation. As previously described, extracorporeal circuits were assembled with the use of a pediatric membrane oxygenator (VP CML Plus: Cobe Cardiovascular, Arvada, Colo) and a roller pump (Cardiovascular Instruments Corp, Wakefield, Mass). The oxygenator and circuitry were primed with lactated Ringer's solution containing dextrose (4.0 g/L), mannitol (4.0 g/L), and porcine heparin (5 U/mL) and circulated at 1.5 L/min with sweep gas flow (95% oxygen, 5% carbon dioxide) at 0.25 L/min. The pH, P_{O_2}, and temperature were continuously monitored and maintained at a pH of 7.35 to 7.45 and a P_{O_2} greater than 150 mm Hg.

Extracorporeal circuit operation and sampling.

After gaining approval from the Human Investigation Committee of Yale University School of Medicine and obtaining informed consent from the subjects, we drew blood (500 mL) over a 5-minute period from healthy volunteers who were receiving no medications. The blood was drawn into a transfer pack (Baxter Healthcare Corp, Deerfield, Ill) containing porcine heparin (5 U/mL final concentration). Anti-C8 or control Mab was added to the transfer pack immediately before addition of the blood to the extracorporeal circuit. As blood was introduced to the circuit reservoir, 400 mL of prime fluid was simultaneously withdrawn to yield a final circuit volume of 700 mL, a mean hematocrit value of 24% ± 4% (standard deviation), and a final Mab concentration of 40 μg/mL. Blood was gently and slowly circulated with prime, and complete mixing was accomplished within 2 minutes; this point was designated as time 0. The circuit flow rate was then accelerated to 1.5 L/min, cooled to 27°C over 5 minutes, and maintained at that temperature for 60 minutes. Then it was rewarmed to 37°C for an additional 30 minutes (90 minutes of total recirculation time), simulating CPB at our institution. Six experiments were performed with anti-C8 Mab at 40 μg/mL and 4 with control Mab at the same final concentration. Blood samples were drawn at 0, 5, 15, 30, 45, 60, 75, and 90 minutes of recirculation. Plasma samples for C3a and C5b-9 were immediately snap-frozen in liquid nitrogen and stored at –70°C until assayed. Plasma samples for neutrophil elastase-antitrypsin complex were anticoagulated with EDTA and similarly snap-frozen. Whole blood samples for flow cytometric studies were immediately fixed in 1% (final concentration) paraformaldehyde in phosphate-buffered saline solution.[17] Additional whole blood samples were drawn into EDTA, 5 mmol/L, at 0, 30, and 90 minutes for a complete blood count and leukocyte differential.

Flow cytometry. Whole blood samples were fixed for 60 minutes at 4°C followed by addition of 1:8 vol/vol of Tris-glycine.[18] Samples were washed twice and resuspended in Tyrode-HEPES buffer, aliquots were incubated with fluorescent Mab at 4°C for 20 minutes, and then washed and resuspended in Tyrode-HEPES buffer for flow cytometric analysis. The percentage of leukocytes with bound platelets and leukocyte activation was determined by labeling samples with (1) fluorescein isothiocyanate–anti-CD45 and phycoerythrin–anti–glycoprotein IIb/IIIa and (2) fluorescein isothiocyanate–anti-CD45 and phycoerythrin–anti-CD11b, respectively. Platelet CD62P expression was determined by labeling with (3) fluorescein isothiocyanate–anti-glycoprotein IIb/IIIa

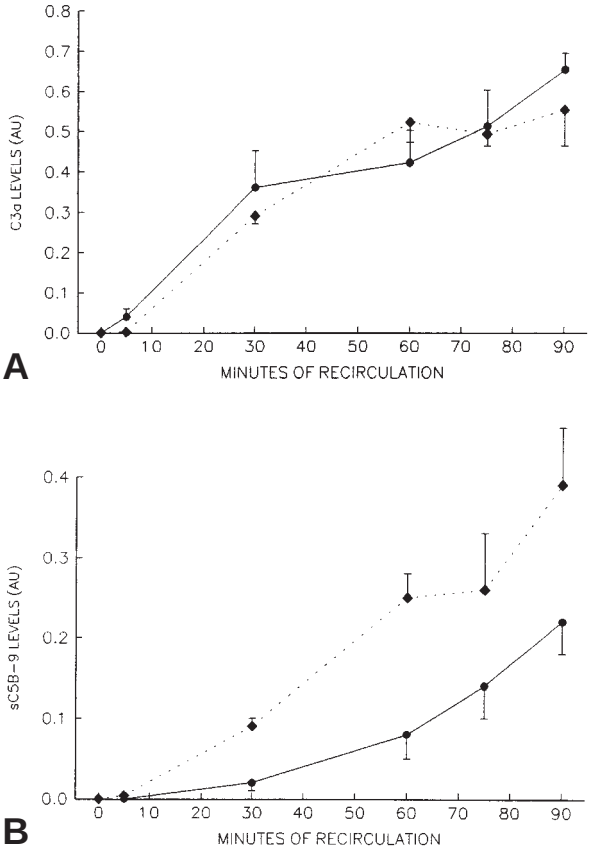

Fig 1. C3a and C5b-9 during SECC. Levels of C3a **(A)** and sC5b-9 **(B)** during SECC were measured in plasma samples taken at the time points shown on the *abscissa.* The mean ± standard error of the mean from 4 control experiments (●) and 6 experiments with addition of the anti-C8 Mab at 40 mg/mL (◆) are shown. Anti-C8 significantly inhibited C5b-9 (*B*, $P = .01$) but not C3a (*A*, $P = .64$) generation.

and phycoerythrin–anti-CD62P.[18] Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, Calif). Leukocyte measurements were carried out by live-gating on fluorescein isothiocyanate–positive, leukocyte-sized events, and PMN and monocyte populations were distinguished on the basis of size, granularity, and differential CD45 expression.[18] Mean CD11b fluorescence and the percentage of leukocyte-platelet conjugates were determined as previously described.[9] Platelet analysis was accomplished by acquisition of fluorescein isothiocyanate–positive, platelet-sized events with the percentage of platelets expressing CD62P determined as previously described.[18] An isotype-matched control Mab was used to set a threshold (99% of events below threshold) for CD62P expression and quantitation of leukocyte-platelet conjugates.

Plasma assays. C3a and C5b-9 levels were measured by enzyme-linked immunosorbent assay kits from Quidel (San Diego, Calif) according to the methods described by the manufacturer. Levels were reported in absorbance units (AU) as in previous work.[15] Neutrophil elastase levels were similarly measured by enzyme-linked immunosorbent assay (E.M. Sciences, Gibbstown, NJ) according to the manufacturer's instructions.

Statistics. C3a and C5b-9 levels are reported as mean ± standard error of the mean of the absolute values. All other results are expressed as a percentage of the individual experiment's time 0 value to minimize the effect of interdonor variability. Thus changes over time of recirculation are expressed as relative changes compared with the starting value and are reported as mean ± standard error of the mean. Two-way analysis of variance for time and antibody effect was performed with GraphPad software (Prism, San Diego Calif).

Results

Complement activation. C3a and sC5b-9 levels were nearly undetectable at baseline. Control SECC (SECC with control Mab) resulted in significant complement activation, with C3a rising from 0.002 ± 0.0001 AU to 0.553 ± 0.12 AU and sC5b-9 increasing from 0.003 ± 0.001 AU to 0.39 ± 0.08 AU after 90 minutes of recirculation (Fig 1, *A* and *B*, $P = .001$ for both). As expected, recirculation after addition of the anti-C8 Mab at 40 μg/mL did not block C3a generation (0.65 ± 0.14 AU at 90 minutes, $P = .64$, Fig 1, *A*). By contrast, sC5b-9 formation was significantly reduced, albeit not completely abrogated, by addition of the anti-C8 Mab, reaching a peak at 90 minutes of 0.22 ± 0.037 AU ($P = .01$ for anti-C8 vs control Mab, Fig 1, *B*).

Platelet activation. The percentage of activated (CD62P-positive) platelets increased significantly during control SECC, peaking at 202% ± 52% of the baseline value after 90 minutes of recirculation ($P = .01$). Anti-C8 Mab addition abrogated this increase, with the percentage of activated platelets peaking earlier during SECC (at 15 minutes) and only reaching 125% ± 13% of baseline ($P = .001$, Fig 2, *A*); the percentage of activated platelets then decreased slightly during the remaining time of recirculation. As another, perhaps more sensitive, indicator of overall platelet activation,[19] the increase in circulating monocyte-platelet conjugates during SECC was also significantly inhibited by C8 blockade, peaking at 153% ± 9% of baseline at 90 minutes compared with 203% ± 51% for control Mab (Fig 2, *B*, $P = .001$). PMN-platelet conjugates also formed during control SECC, peaking at 306% ± 112% after 90 minutes of recirculation (Fig 2, *C*). Inhibition of formation of PMN-platelet conjugates by the anti-C8 Mab did not reach statistical significance, with levels peaking at 234% ± 54% after 60 minutes of recirculation ($P = .057$).

Cell counts. The platelet count during control SECC was $130 \times 10^3/\mu L \pm 11 \times 10^3/\mu L$ at time 0; platelet

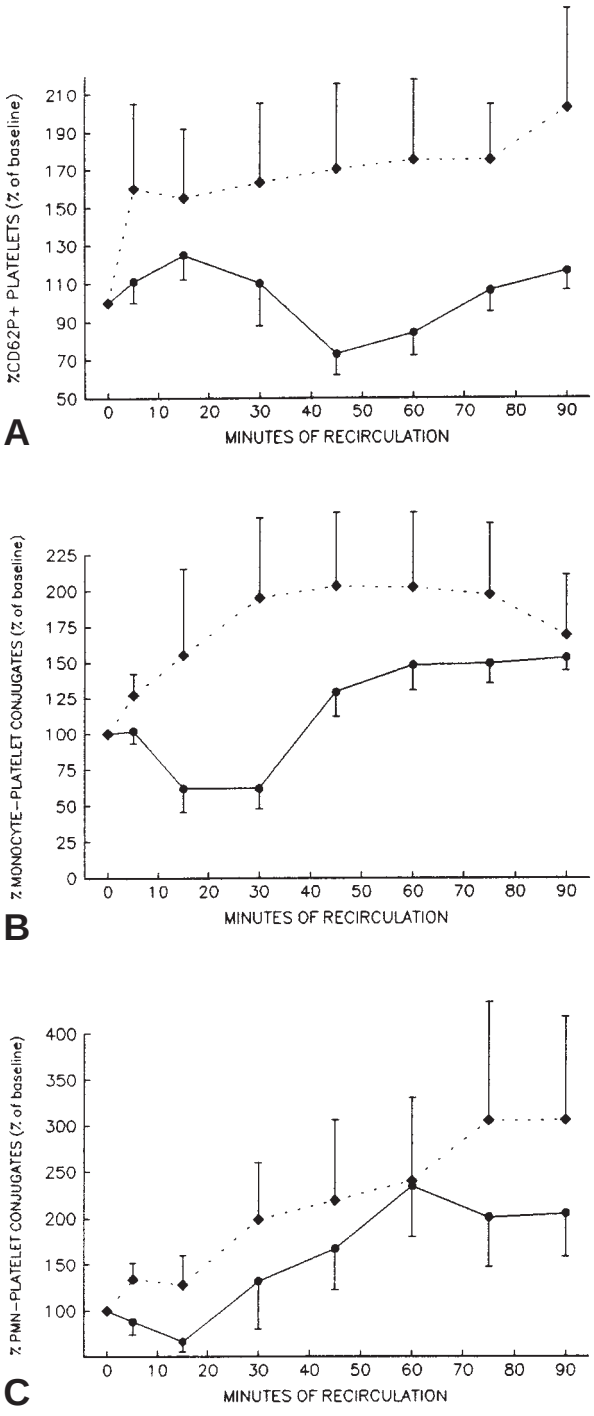

Fig 2. CD62P-positive platelets and platelet-leukocyte conjugate formation during SECC. The percentage of circulating CD62P-positive platelets **(A),** the percentage of monocytes binding platelets **(B),** and the percentage of PMN binding platelets **(C)** during SECC were measured in whole blood samples taken at the time points shown on the *abscissa* and expressed as a percentage of the time 0 value. The mean ± standard error of the mean from 4 control experiments (◆) and 6 experiments with addition of the anti-C8 Mab at 40 μg/mL (●) are shown. Anti-C8 significantly inhibited the increase in CD62P-positive platelets (P = .001) and the formation of monocyte-platelet conjugates (P = .001) but not the increase in PMN-platelet conjugates (P = .057).

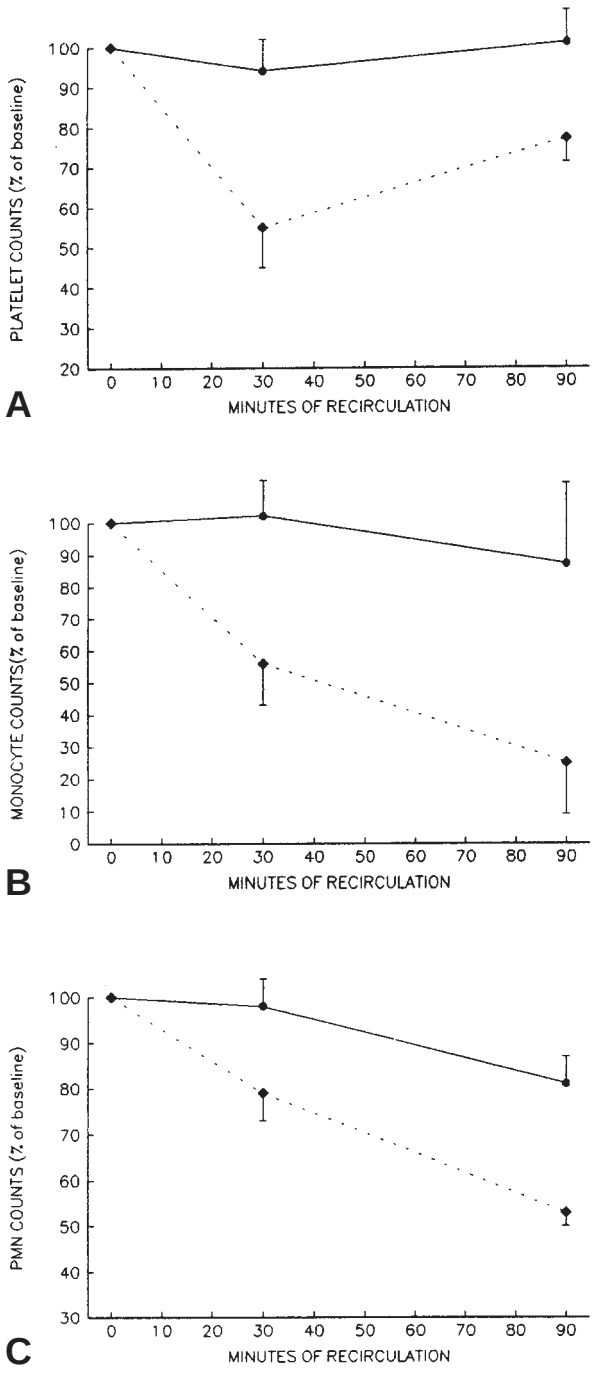

Fig 3. Platelet, monocyte, and PMN counts during SECC. Platelet **(A),** monocyte **(B),** and PMN **(C)** counts during SECC were measured in whole blood samples taken at the time points shown on the *abscissa* and expressed as a percentage of the time 0 value. The mean ± standard error of the mean from 4 control experiments (◆) and 6 experiments with addition of the anti-C8 Mab at 40 μg/mL (●) are shown. Anti-C8 significantly inhibited the decrease in platelets, monocytes, and PMN (P = .01 for all 3) during SECC.

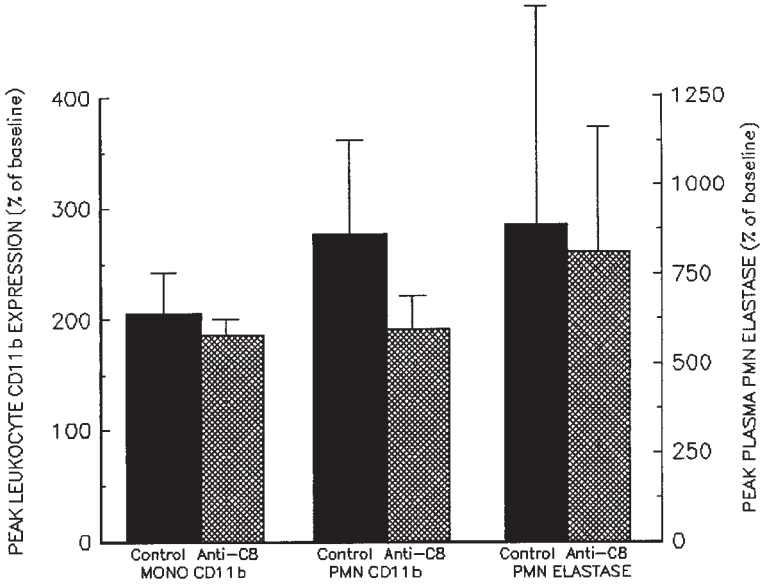

Fig 4. Leukocyte activation during SECC. Peak expression of CD11b on monocytes and PMNs in whole blood samples *(left axis)* and the PMN elastase in plasma *(right axis)* are expressed as a percentage of the time 0 value. The mean ± standard error of the mean from 4 control experiments *(solid bars)* and 6 experiments with addition of the anti-C8 Mab at 40 μg/mL *(hatched bars)* are shown. Anti-C8 did not inhibit the increase in monocyte CD11b, PMN CD11b, or PMN elastase (P = .15 for all) during SECC.

counts decreased to a nadir of 55% ± 10% of the baseline value after 30 minutes of recirculation and then increased to 77% ± 6% with rewarming. This decrease was completely inhibited by the anti-C8 Mab, beginning with a platelet count of $133 \times 10^3/\mu L \pm 18 \times 10^3/\mu L$ at time 0 and falling to only 94% ± 8% of the baseline value after 30 minutes of recirculation (P = .01, Fig 3, *A*). Circulating monocytes also decreased during control SECC, beginning recirculation at 373 ± 11 cells/μL and reaching a nadir of 25% ± 6% of baseline after 90 minutes. Addition of Anti-C8 Mab produced significantly greater preservation of monocyte counts, beginning recirculation at 315 ± 55 cells/μL and falling to only 87% ± 25% after 90 minutes (P = .01, Fig 3, *B*). Similarly, the anti-C8 Mab preserved PMN counts during SECC, beginning with $2.1 \times 10^3 \pm 0.2 \times 10^3$ cells/μL and decreasing to a nadir of only 81% ± 3% of the baseline value (P = .01, Fig 3, *C*), compared with a time 0 value of $3.2 \times 10^3 \pm .9 \times 10^3$ cells/μL and decreasing to 53% ± 6% over the same period during control SECC.

Leukocyte activation. Control SECC activated both PMNs and monocytes, with CD11b levels increasing to 251% ± 66% and 206% ± 36% of their baseline values, respectively (P = .01 for both), similar to previous studies.[10] Neutrophil elastase levels increased to 889% ± 614% of baseline values after 90 minutes of recirculation (P = .01). Addition of the anti-C8 Mab to SECC had no effect on leukocyte activation; neutrophil and monocyte CD11b increased to 187% ± 14% and 191% ± 30%, respectively (P = .2 for both); similarly, neutrophil elastase levels rose to 812% ± 353% of the baseline value (P = .50, Fig 4).

Discussion

This investigation has demonstrated that selective blockade of the C8 complement component successfully decreases sC5b-9 formation during SECC. Inhibition of MAC formation was associated with a significant reduction in platelet activation as measured by both the percentage of CD62P-positive platelets and monocyte-platelet conjugate formation. Formation of the latter complex is predominantly dependent on activation-induced platelet surface expression of CD62P, an adhesive ligand for monocytes, and to a lesser extent, PMN,[9] and thus supports the conclusion that platelet activation on SECC is primarily caused by MAC formation. These findings are also consistent with the in vitro demonstration of C5b-9–induced CD62P expression.[20] Furthermore, abrogation of the decline in the number of circulating platelets during SECC by anti-C8 is likely also a result of reduced

platelet activation. The role of temperature in MAC-induced platelet activation was not directly examined in this study; however, the increases in circulating CD62P-positive platelets and monocyte-platelet conjugates occurring early during the hypothermic phase of control SECC support the finding[21] that hypothermia does not abrogate the stimulatory effects of CPB on adhesion molecules.

In contrast to platelet activation, selective blockade of MAC formation did not inhibit either monocyte or PMN activation, as measured by up-regulation of the adhesion receptor CD11b and neutrophil elastase release. Complement activation, along with contact activation–induced mediators, for example, kallikrein,[22] contributes to the generalized inflammatory response to CPB, but the link between specific complement components and cellular activation pathways has been difficult to ascertain. Previous work from our laboratory demonstrated that anti-C5 blockade on SECC inhibited C5a and C5b-9 formation but did not check C3a formation. In addition to inhibiting platelet activation and formation of platelet-leukocyte conjugates, blockade of both C5a and C5b-9 formation prevented PMN activation. By contrast, in the current study, selective C5b-9 inhibition did not affect PMN activation. These data suggest that C5a, rather than C5b-9, is responsible for the complement-related PMN activation induced by SECC. This is consistent with in vitro studies in which C5a is a potent activator of PMN.[11] The effects of C5b-9 on leukocytes appear to be more subtle. Nucleated cells are relatively resistant to the lytic effects of complement membrane attack[23]; however, nonlytic C5b-9 levels can induce release of reactive oxygen metabolites from PMNs,[24] creating the potential for additional proinflammatory effects. These latter effects, however, may not be detectable in the setting of the more profound levels of PMN activation induced when C5a formation is unopposed, as in the present study.

It is important to note one caveat: because we were unable to completely abrogate C5b-9 formation, it remains possible, albeit unlikely, that the small amounts of C5b-9 produced in the presence of the anti-C8 Mab were sufficient to activate PMNs. The incomplete blockade of MAC may be a function of the particular Mab used or may be a universal finding with any inhibitor of C8 cleavage. The Mab dosage did not appear to be the limiting factor, given that doubling the anti-C8 dose did not additionally improve MAC inhibition (data not shown). Therefore, although these results demonstrate that inhibition of platelet activation by terminal complement blockade is predominantly caused by C5b-9 rather than C5a, it is not possible to determine whether C5b-9 generation contributes in more subtle ways to PMN activation. Nevertheless, on the basis of the ability of in vitro C5a to potently activate PMNs,[11] and the current findings, we believe C5a to be one of the major inducers of PMN activation in the SECC setting. Blockade of C5a formation and contact activation–induced kallikrein production are promising strategies for reducing the inflammatory response to CPB.

MAC inhibition also preserved the total number of circulating platelets, monocytes, and PMNs compared with control Mab runs. Although we expected that reduced platelet activation would limit platelet adhesion to the circuit[25] and preserve platelet numbers, the surprisingly effective preservation of leukocyte counts, despite continued leukocyte activation, may suggest important features of the pathophysiology of leukocyte-biomaterial interaction. Preservation of the absolute numbers of circulating leukocytes could be an indirect result of reduced platelet activation in the presence of MAC inhibition. Data from our laboratory and others[26,27] have suggested that leukocyte adhesion to a biomaterial surface can occur as the result of "bridging" by platelets bound both to the material and to leukocytes. Fibrinogen binds rapidly to the CPB circuit; activated platelets may adhere to the bound fibrinogen via glycoprotein IIb/IIIa[27]; monocytes and PMNs may then bind via CD62P to these platelets and consequently be lost to the circulation. Since circulating platelet-leukocyte conjugates were reduced by anti-C8, it is possible that a similar event occurs on the circuit itself, although other explanations such as decreased mediator release by activated platelets are also possible.

It is not known whether selective inhibition of C5b-9 formation would be of benefit in clinical settings necessitating extracorporeal circulation. In theory, specific inhibition of platelet activation by the MAC may reduce procoagulant activity in the perioperative period, because MAC assembly on the platelet surface causes phosphatidylserine exposure, produces procoagulant platelet microparticles,[6] and enhances platelet "procoagulant activity" in vitro.[20] The activated platelet may also increase monocyte procoagulant activity through CD62P-mediated induction of tissue factor.[28] Thus selective inhibition of MAC formation might be appropriate for circumstances in which it is desirable for PMNs to remain responsive to complement, but at the same time limiting the procoagulant potential of platelets and monocyte-platelet conjugates. In the setting of CPB, however, the additional reduction of proinflammatory PMNs through complement and/or contact

pathway inhibition is desirable to reduce "reperfusion injury" physiology. Blockade at C5b-9 alone is not likely to block this physiology and has the potential to worsen any inflammatory component by preserving greater numbers of activated PMNs in the circulation.

In summary, selective inhibition of C5b-9 generation during SECC resulted in significant inhibition of platelet activation and activation-dependent monocyte-platelet conjugate formation. This inhibition also allowed greater preservation of absolute numbers of circulating platelets, as well as PMNs and monocytes. By contrast, C5b-9 inhibition did not produce any decrease in PMN or monocyte activation, suggesting at best a minor role for this complement component in leukocyte activation during SECC. This study also suggests that platelet activation and/or MAC may be responsible in part for the loss of circulating PMNs and monocytes to the extracorporeal circuit.

REFERENCES

1. Parker D, Cantrell J, Karp R, Stroud R, Digerness S. Changes in serum complement and immunoglobulins following cardiopulmonary bypass. Surgery 1972;71:824-7.
2. Steinberg J, Kapelanski D, Olson J, Weiler J. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1993;106:1008-16.
3. Williams L, Burke W, Steele R. Complement: function and clinical relevance. Ann Allergy 1988;60:293-300.
4. Wiedmer T, Esmon CT, Sims PJ. Complement proteins C5b-9 stimulate procoagulant activity through the platelet prothrombinase. Blood 1986;68:875-80.
5. Wiedmer T, Esmon CT, Sims PJ. On the mechanism by which complement proteins C5b-9 increase platelet prothrombinase activity. J Biol Chem 1986;261:14587-92.
6. Wiedmer T, Sims P. Participation of protein kinases in complement C5b-9 induced shedding of platelet plasma membrane vesicles. Blood 1991;78:2880-6.
7. Hamburger SA, McEver RP. GMP-140 mediates adhesion of stimulated platelet to neutrophils. Blood 1990;75:550-5.
8. Rinder C, Bonnert J, Rinder H, Mitchell J, Ault K, Hillman R. Platelet activation and aggregation during cardiopulmonary bypass. Anesthesiology 1991;74:388-93.
9. Rinder C, Gaal D, Student L, Smith BR. Platelet-leukocyte activation and modulation of adhesion receptors in pediatric patients with congenital heart disease undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1994;107:280-8.
10. Rinder C, Bonan J, Rinder H, Mathew J, Hines R, Smith BR. Cardiopulmonary bypass induces leukocyte-platelet adhesion. Blood 1992;79:1201-5.
11. Tonnesen M, Anderson D, Springer T, Knedler A, Avdi N, Henson P. Adherence on neutrophils to cultured human microvascular endothelial cells: simulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. J Clin Invest 1989;83:6337-46.
12. Hattori R, Hamilton KK, Fugate RD, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. J Biol Chem 1989;264:9053-60.
13. Wachtfogel Y, Musial J, Jenkins B, Niewiarowski S, Edmunds LH Jr, Colman RW. Loss of platelet α_2-adrenergic receptors during simulated extracorporeal circulation: prevention with prostaglandin E^1. J Lab Clin Med 1985;105:601-7.
14. Kappelmayer J, Bernabie A, Edmunds LH Jr, Edgington T, Colman RW. Tissue factor is expressed on monocytes during simulated extracorporeal circulation. Circ Res 1993;72:1075-81.
15. Rinder CS, Rinder HM, Smith BR, et al. Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. J Clin Invest 1995;96:1564-72.
16. Rollins SE, Matis LA, Springhorn JP, et al. Monoclonal antibodies directed against human C5 and C8 block complement-mediated damage of xenogeneic cells and organs. Transplantation 1995;60:1284-92.
17. Rinder HM, Bonan J, Rinder CS, Ault KA, Smith BR. Activated and unactivated platelet adhesion to monocytes and neutrophils. Blood 1991;78:1760-9.
18. Rinder HM, Bonan J, Rinder CS, Ault KA, Smith BR. Dynamics of leukocyte-platelet adhesion in whole blood. Blood 1991;78: 173-6.
19. Leo E, Gemmell C. Minimal P-selectin content required for platelet-leukocyte adhesion. Blood 1993;82(Suppl 1):621a.
20. Sims PJ, Wiedmer T. Induction of cellular procoagulant activity by the membrane attack complex of complement. Semin Cell Biol 1995;6:275-82.
21. Boldt J, Osmer C, Linke LC, Gorlach G, Hempelmann G. Hypothermic versus normothermic cardiopulmonary bypass: influence on circulation adhesion molecules. J Cardiothorac Vasc Anesth 1996;10:342-7.
22. Kappelmayer J, Bernabei A, Gikakis N, Edmunds LH Jr, Colman RW. Upregulation of Mac-1 surface expression on neutrophils during SECC. J Lab Clin Med 1993;121:118-26.
23. Morgan BP. Complement membrane attack on nucleated cells: resistance, recovery and non-lethal effects. Biochem J 1989; 2641-14.
24. Campbell AK, Morgan BP. Monoclonal antibodies demonstrate protection of polymorphonuclear leukocytes against complement attack. Nature 1985;317:164-6.
25. Addonizio VP, Macarak EJ, Niewiarowski S, Colman RW, Edmunds LH Jr. Preservation of human platelets with prostaglandin E1 during in vitro simulation of cardiopulmonary bypass. Circ Res 1979;44:350-7.
26. Palabrica T, Lobb R, Furie BC, et al. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature 1992;359:848-51.
27. Musial J, Niewiarowski S, Hershock D, et al. Loss of fibrinogen receptors from the platelet surface during simulated extracorporeal circulation. J Lab Clin Med 1985;105:514-22.
28. Amirhovsravi A, Alexander M, May K, et al. The importance of platelets in the expression of monocyte tissue factor antigen measured by a new whole blood flow cytometric assay. Thromb Haemost 1996;75:87-95.